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Different Originating Cells Underlie Intertumoral Heterogeneity in Lung neuroendocrine Tumors

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Summary

Studies in genetically engineered mouse models of neuroendocrine lung cancer suggest that differences in cells of origin underlie subtype variations in this class of cancers. These findings highlight the concept that the same driver mutations introduced into different cells of origin lead to tumors with the same histology but dramatically different metastatic programs and potentially different therapeutic responses.

The primary site of a tumor, combined with its “lineage” and its histology, has determined clinical decisions for decades. Recently, molecular analyses of lung cancers of the same lineage and histology, such as EGFR mutation status and PD-L1 expression, have also affected care decisions. It is also becoming evident that tumors of the same lineage and histology, that have similar driver mutations, may have important clinical differences depending on the cell of origin from which the tumor arose. In this issue of *Cancer Discovery*, Yang and colleagues utilize a genetically engineered mouse model (GEMM) of lung cancer to identify different cells of origin for small cell lung cancer (SCLC) that exhibit distinct metastatic properties (1). In patients, SCLC is an aggressive, highly metastatic neuroendocrine (NE) cancer in urgent need of detailed understanding of its mechanistic underpinnings and, from these, the identification of vulnerabilities that can be therapeutically targeted (2). Yang and colleagues demonstrate distinctions in GEMMs for SCLC generated by targeting different populations of putative tumor-originating cell types in the lung. These distinctions may represent subtype-specific variations being detected in

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SCLC patient populations through variations in histology, NE gene signatures, chromatin landscape, and responses to treatment.

SCLC occurs in smokers and has a 5-year survival rate of <7%. A clinical diagnosis of SCLC is usually made after a histologic examination and IHC detection of NE markers in a tumor biopsy. Molecular analyses of SCLC have shown that in essentially all cases, inactivation of *RB1* and *TP53* tumor suppressor genes has occurred (3). Thus, in designing the first GEMMs for SCLC, these genes were deleted broadly in the respiratory epithelium using mice with floxed alleles of the tumor suppressor genes and adenovirus containing CMV-Cre. Subsequently, modifications in this design were reported including deletion of an additional gene, *Rbl2* (p130), to speed up the appearance of tumors (from ~12 months in the original *Trp53;Rb1* double knockout to ~6 months with the *Trp53;Rb1;Rbl2* triple knockout, or TKO; ref. 4). Further elegant experiments to uncover the cell type of origin for these NE tumors replaced CMV in the virus with cell type-specific promoters, such as the neuronal-specific CGRP promoter, and revealed lung CGRP⁺ NE cells as a cell of origin for SCLC (5). These models represent the major subtype of SCLC characterized by high levels of the lineage transcription factor ASCL1 and a strong NE gene signature. By comparing the generation of primary and metastatic NE tumors in these related models, Yang and colleagues uncover differences not previously appreciated. These findings highlight the key concept that the originating cell type that incurs the tumor-initiating mutations has a lasting influence on the metastatic program and, by inference, therapeutic response of the cancer (Fig. 1). Furthermore, this study reveals intertumoral heterogeneity even within the ASCL1^{hi} subtype of SCLC.

The emerging picture in SCLC is that there are multiple subgroups, all with characteristic inactivation of *RB1* and *TP53*, but with distinctions in gene expression. A currently accepted model has ASCL1^{hi} NE^{hi} tumors, representing the major SCLC subtype, arising from *RB1* and *TP53* mutations in a lung CGRP-expressing NE cell. Increasing our understanding of SCLC heterogeneity, a recent report implicated a rare chemosensory cell, the tuft cell, as an originating cell for a different subset of SCLC, the ASCL1^{lo} NE^{lo} (6). Beyond these subtypes, additional intertumoral heterogeneity in SCLC populations is recognized but lacks a biological explanation. This heterogeneity is hypothesized to arise from as-yet-unidentified originating cell types that follow distinct developmental programs in the lung and/or have additional mutations, such as amplification of an MYC gene (7). Yang and colleagues provide evidence for a CGRP-negative epithelial cell in the mouse lung that can efficiently progress to a CGRP⁺ NE tumor when *Trp53* and *Rb1* are lost (Fig. 1). Driving Cre in known non-NE cell types in the lung including the secretory population expressing CC10 (*Scgb1a1*) or the alveolar type II population expressing SPC (*Stfpc*), individually or in combination with the CGRP population, is not sufficient to replicate the tumor formation seen with CMV-Cre. Thus, the existence, but not the identity, of a lung CGRP-negative cell population that can progress to an NE⁺ lung cancer is implicated.

Lineage plasticity is an interesting concept in cancer biology. In the lung, it has been shown that some non-small cell lung carcinomas (NSCLC), a non-NE lung tumor with different cellular origins and driver mutations from SCLC, switch from an NSCLC to an SCLC phenotype to evade tyrosine kinase receptor inhibition (8). Lineage plasticity has been

observed in other cancers as well, such as prostate cancer, particularly when under environmental pressure such as a drug treatment. Here, as a mechanism of resistance to antiandrogen therapies, tumor cells switch to an NE phenotype by inactivating *TP53* and *RB1* and upregulating the transcriptional regulator SOX2 (9). It is worth noting that SOX2 is also present in proximal progenitors in the lung during development, and analogous to the plasticity observed in prostate cancer, the SCLC CMV-TKO model may delete *Trp53* or *Rb1* in a SOX2⁺ cell that retains lineage plasticity. Alternatively, the loss of the tumor suppressors could cause an otherwise non-NE cell to gain SOX2, or some other epigenetic regulator, increasing plasticity and allowing it to progress to an NE proliferative tumor. Yang and colleagues provide evidence for either a cancer-originating cell that normally has the potential to differentiate toward the NE lineage, or one that gains plasticity after loss of *Trp53*;*Rb1*. Further studies are required to distinguish between these possibilities.

In addition to highlighting concepts of heterogeneity and plasticity in SCLC subtypes, this study provides a useful characterization of primary tumors and metastases from the GEMMs that are widely used in the SCLC research field. Because tumors from these models are not identical, it is important to be aware of their similarities and differences that may affect design and interpretation of experiments. As noted above, the SCLC GEMMs have tumors arising from *Trp53*, *Rb1*, and *Rbl2* loss across broad cell types (CMV-TKO) compared with NE cell-specific loss of the tumor suppressors (CGRP-TKO). Prior to the current study, distinctions in SCLC tumors arising in these related GEMMs were not appreciated because both models develop histologically similar tumors that express NE markers. Furthermore, metastases arise within a similar timeframe and exhibit a similar histology. With the more detailed comparison cataloged here, however, important differences were noted including (i) the efficiency of tumor formation (CMV-TKO SCLC are substantially more numerous), (ii) differences in expression of the transcription factor NFIB in metastases (NFIB is seen only in the CMV-TKO metastases), (iii) global differences in gene-expression programs, and (iv) primary tumor location in the lung (CGRP-TKO are restricted to proximal airways, whereas CMV-TKO can also be found in distal airways). Thus, a critical question is whether one, or both, of these mouse models best represent human SCLC. We argue that both models are clinically relevant to the human disease and represent different SCLC subtypes. First, the heterogeneity in NFIB expression seen in metastases from the GEMMs is represented in patients with SCLC, with about 50% of patient tumors containing low or no NFIB. This also makes an important point that NFIB plays a significant role in the metastasis of some, but not all, SCLC subtypes (10). Second, 85% to 95% of human primary SCLCs are located in the proximal airways, and this is reflected in the CGRP-TKO model. However, 5% to 15% of human primary SCLC tumors are found in more peripheral regions, and this is seen only in the CMV-TKO model. Although further studies are needed for a better understanding of these differences, it appears that both mouse models have important relevance for human SCLC research.

The current work shows the importance of linking the cell of origin of an SCLC to its biological behavior. We also need to know if this origin influences an individual SCLC's response to therapy and development of therapy resistance. Other important questions include: how "plastic" is the program arising from the cell of origin; are all cells in the same tumor subtype lineage equally susceptible to malignant transformation; is there information

in the cells of origin that can be used for screening for early diagnosis, as prevention targets, or as therapeutic targets for SCLC? One of the main challenges in SCLC research is the scarcity of patient samples because most diagnoses and clinical decisions are based on fine-needle aspirates or small biopsies. Therefore, mouse models including GEMMs, patient-derived xenografts, as well as human SCLC cell lines have been invaluable tools to advance our understanding of SCLC to identify biomarkers for patient stratification and relevant targets for therapy development. Yang and colleagues have provided compelling evidence for important variations in currently used GEMMs that contribute additional depth to these clinically useful tools. These results call for continued probing of GEMMs to identify all potential cells of origin for SCLC. In the broader context, clinical decisions and translation based on lineage, histology, and driver mutations need to be refined to include methods to identify the exact cell of origin of otherwise similar tumors and to determine the clinical implications of such origin differences.

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Figure 1.

Cell of origin in SCLC GEMMs influences tumor generation, genetic characteristics, and metastasis. Loss of *TP53* and *RB1* are defining mutations in SCLC. **A**, Mouse models for SCLC involve deleting *Trp53*, *Rb1*, and the *Rb1*-related *Rbl2* (*p130*) by introduction of virus expressing Cre to the respiratory epithelium (4). Use of the CMV promoter directs Cre broadly in diverse lung cell types whereas the CGRP promoter directs Cre to rare NE cells in the proximal airways. **B**, Both models result in histologically similar primary NE tumors; however, the CMV-Cre model generates substantially more tumors. **C**, An additional distinction is that metastatic tumors arising from the different models have differential requirement for the transcription factor NFIB, and in comparison to metastases from CGRP-Cre-driven tumors, the CMV-Cre-driven tumors have a more neural developmental gene signature (1). How well these models represent variant subtypes seen in patient SCLC populations, and their distinctions in response to therapies, will have to await future analyses. Pale yellow cells indicate tumor cells that arise from lineage marker-negative cells that progress to a CGRP-positive, NE⁺ phenotype. These tumor cells have similarities to those arising from CGRP-positive cells but are not identical in gene expression and chromatin landscape.